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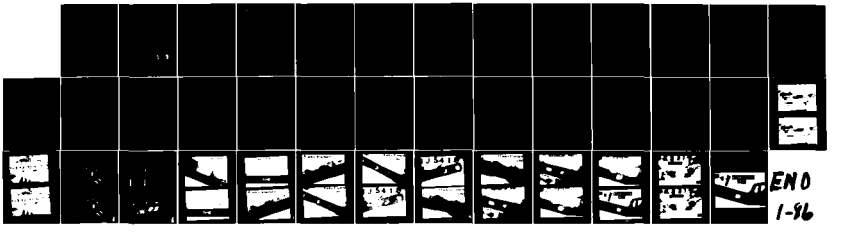

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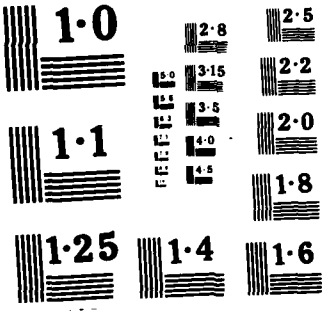
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# A Biodegradable Implant for Restoring Bone Discontinuity Defects in Dogs

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and

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Daniel M. Laskin, DDS
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Dear Dr. Laskin:

Dr. Schmitz and I have submitted our article "A Biodegradable Implant for Restoring Bone Discontinuity Defects" for consideration in the Journal of Oral & Maxillofacial Surgery. Please address all correspondence to me at the address shown below.

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## ABSTRACT

A copolymer (polylactic acid: polyglycolic acid) was combined with a proteolipid (PL) and the resulting implant complex was rigidly fixed into discontinuities in the mandibles of 25 adult, foxhound dogs. Identically prepared control discontinuities in contralateral sites in the same animals did not receive the complex and were rigidly fixed. At 4, 8, 12, 24, 40 weeks the dogs were sacrificed and implant and controls were prepared for histomorphometry. Histomorphometric evaluation revealed there was a linear increase of bony reparative elements in the implants over 40 weeks that exceeded those of the nontreated control sites. The copolymer-PL implant, therefore, may provide an alternative to autogeneic and allogeneic bone substances.

## INTRODUCTION

A variety of materials are described in the literature for bone repair, replacement, and augmentation. Poly-alpha-hydroxy acids have been investigated in our laboratory because of their favorable bone repair characteristics. *Polyglycolic acid* (PGA) and polylactic acid (PLA) are two examples of poly-alpha-hydroxy acids that were initially formulated and described as biodegradable suture materials.[1-3] Configurations of PLA and PGA as either homopolymers or copolymers also have been used experimentally for osseous repair.[4-9] The properties of PLA and PGA that make these agents attractive as bone repair materials are biodegradability and biocompatibility. These properties may be exploited when PLA and PGA are used as carriers or vehicles for known or putative bone repair substances. It was hypothesized by Hollinger that the addition of a specific proteolipid (PL) -- a certain acidic phospholipid -- to the copolymer of PLA and PGA, that the osseous repair cascade would be enhanced because of the avidity of the PL for calcium-phosphate complexes.

It has been demonstrated by several workers that certain types of acidic phospholipids can induce hydroxyapatite formation both <u>in vitro</u> and

*in vivo*.[10-13] The importance of membrane component phospholipids in signal transmission for hormones, neurotransmitters, and growth factors once again emphasizes the significant contribution of these agents in complex physiological processes. The purpose of this study, therefore, was to determine if the combination of copolymer PLA:PGA and PL could enhance the bone repair rate in mandibular discontinuities in dogs.

## MATERIALS AND METHODS

### Preparation of the Implant Material

The acidic phospholipid (PL) and copolymer were prepared as previously described and the properties of these agents were identical to those in the earlier reports.[9, 13, 16] The cured implants were 20 mm x 17 mm x 8 mm and weighed 2.5 $\pm$ 0.3 gms. Implants were sterilized in ethylene oxide (30° C, 4-5 psi, 6 hrs) and degassed to remove all residual ethylene oxide and possible ethylene glycol and ethylene chlorohydrin residuals.

## Preparation of Experimental Animals

### Laboratory Animals

Prior to the study, 25 adult (60-70 lbs), random sex, foxhound dogs were obtained and conditioned for at least two weeks. Skeletal maturity authenticating adulthood was determined by observing closure of epiphyses of the distal femur and proximal tibia on roentgenographic films. Animals were verified as being healthy by routine physical examinations and accepted veterinary diagnostic testing.

### Anesthesia

All animals were kept NPO for 24 hours before surgery. Benzathine penicillin (150,000 units) was given 24 hrs before surgery and preoperatively, acepromazine (5 mg) and atropine (1 mg) were administered intramuscularly and subcutaneously, respectively. An IV line was established in the left hind leg and Ringer's lactate (1 cc/min) was infused during the entire procedure until the animal was responsive. After induction of anesthesia with sodium pentothal (20 mg/kg, IV), animals were intubated and maintained at a surgical plane of anesthesia with 2 % halo-

thane and $N_2O/O_2$ 50:50 (1 liter/min).

## Surgery

### Pre-extraction Resection

The 25 dogs were divided into two groups: 18 for extraction of teeth in the resection site and 7 without extraction of teeth . The extraction group had teeth distal to the canine and up to the second molar extracted bilaterally and in both arches. Interocclusal dimensions were satisfied by retention of the distal molars. Alveoloplasties in the extraction sites were performed with rotary and hand instruments and copious sterile water irrigation and soft tissue was closed in layers with 4-0 Dexon®. Antibiotic coverage consisted of 150,000 units of Bicillin® for 5 days. Both groups of animals were maintained on a soft dental diet for the duration of the study.

### Resection

Following a surgical scrub of the shaved skin with povidone iodine and methanol, the animals were draped and the mandible was approached through an external submandibular incision. The mandible was

degloved facially and lingually in the area of the second carnasal tooth. Following ligation of the neurovascular bundle proximally and distally, a 17 mm discontinuity defect (cortex to cortex) was created using a highspeed drill and copious sterile water as an irrigant. All animals received identical bilateral defects in the body of the mandible. The right side (experimental) received a 17 mm x 17 mm x 8 mm copolymer-PL implant while the left side served as a control (no implant). The implant was secured with 21 gauge stainless steel wire. The proximal and distal mandibular fragments were fixated on each side with a tubular stainless steel bone plate and four self-tapping screws (Fig. 1a and b). A water-tight seal of the intraoral mucosa was accomplished with 3-0 Dexon® and the submandibular tissues were closed in layers with 4-0 Dexon®. Antibiotic coverage and the soft dental diet were described in the pre-extraction section.

Necropsy Schedule

At five post-implantation times (4, 8, 12, 24, and 40 weeks) five animals were euthanatized with T-61®. The number of extraction to nonextraction animals varied from 3:2 to 4:1, respectively (Table).

Preparation of Specimens for Histomorphometric Analysis

At the time of necropsy, experimental and control areas were surgically removed en bloc. A 100 micron slice was retrieved from the center of the specimen (frontal plane) using a Buehler Isomet Low Speed Saw and the slice was placed into 70% ethanol, embedded in polymethyl methacrylate, sectioned at three and one-half to four microns and stained with a modification of Masson-Goldner trichrome stain to facilitate histomorphometric analysis (Fig. 2).[16] A 22 mm x 10 mm window was placed over each prepared slide specimen to standardize and define the assessment perimeter. Using a 1.25X objective and an eyepiece greticle in a Zeiss 10X eyepiece, a grid was superimposed over each specimen that defined the fields to be measured within that perimeter. Therefore, within a 220 $mm^2$ area (22 mm X 10 mm) it was possible to measure two fields that included approximately 50 grid squares per field (Figs. 3a and b). Measurements were made of the two-dimensional bony trabeculum in each field using a Zeiss Image Analysis System with Osteoplan™ (version 4.1) (Fig. 4) to derive three-dimensionality, that is: the first order derived quantity of trabecular volume (TV) ($mm^3$ trabecular bone/$mm^3$ bone tissue).

## DATA ANALYSIS

For each necropsy time (4, 8, 12, 24, and 40 weeks), means and standard deviations (across five animals after taking the mean of the duplicate measurements) were computed for implants and controls. Overall differences in mean TV between implants and controls and effects of necropsy time were tested using analysis of variance procedures for a two factor repeat measure design. (Implant vs. control is a repeat measure factor on the same dog, whereas necropsy time is not a repeat measure.) The paired t-test was used for making individual comparisons of implant and control means at each necropsy time.

## RESULTS

### Clinical and Radiographic

Clinical problems developed in eight dogs that included localized osteomyelitis, abscess formation, and loosening and/or loss of fixation devices. Six of the eight dogs were from the group that did not have pre-ablation extraction. All eight dogs were treated successfully by

standard procedures and, therefore, they were able to continue in our study. Overall, the clinical observations during the study were relatively unremarkable.

Radiographically, bone regeneration was noted in four out of five dogs in the implant group at four weeks. (The implant is radiolucent, therefore, radio-opacities are not indicative of the copolymer-PL.) A gradual progression of bony regeneration at the implant sites produced complete osseous bridging by 40 weeks (Figs. 5a-9a). At control sites, radio-opacities were observed in two out of five animals at four weeks. Only one animal failed to produce a complete osseous bridge by 40 weeks (Figs. 5b-9b).

Histomorphometric Analysis

Fig. 10 summarizes the effects of treatment (implant vs. control) and necropsy time on TV. Analysis of variance demonstrated an overall effect of treatment with differences between temporal groups. For both implant and control, mean TV increased over time, reaching a plateau at approximately 24 to 40 weeks. For each necropsy time (temporal group), the TV of the implants exceeded the controls ($p < 0.05$ using the paired t-test).

## DISCUSSION

In a previous report, it was mentioned that the copolymer of PLA:PGA (50:50) combined with a PL stimulated the early phases of osseous repair in rat long bone.[16, 17] In this study, we determined that osseous repair in discontinuity defects in dogs' mandibles treated with the same combination, healed more rapidly than untreated defects and that the material was completely tissue tolerant.

The dog experiment involved a bony wound that was not a critical size defect (CSD). (The importance of a CSD has been reviewed and described for bone repair studies.[18]) We were not interested in an "all or none" phenomenon; rather, what was the effect of the alloplastic material on the rate of bone repair. (We define rate as being the amount of bone -- Trabecular Volume -- developed over time.) Because there is no unequivocal mandibular CSD for dogs, the authors completed an experimental evaluation of a convenient dog model for mandibular discontinuities. This model, coupled with a standardized radiographic technique in the adult dog is recommended as a test system for bone repair materials.[18] Furthermore, because of the post resection complications when teeth are not extracted, we advocate extrac-

tion of teeth in the planned resection site followed by at least six weeks of healing before ablation.[18]

Previous publications have mentioned how the alloplastic copolymer benefits from the inclusion of PL.[13, 16, 17] Despite the fact that unembellished copolymer engendered a more rapid rate of bone repair than in untreated control wounds in endochondral bone in rats, we did not opt to prepare unembellished copolymer implant blocks for the dogs.[9] The choice of the copolymer-PL combination was made because of the data previously derived from histomorphometric evaluations of the two types of implants (copolymer and copolymer-PL).[16] It was determined that the combination was clearly superior to the plain copolymer. We could not use the PL by itself because it has a paste-like consistency.

The PLA:PGA copolymer appears to be a suitable carrier vehicle for bone formation agents. The use of homopolymers and copolymers of PLA and PGA for bone repair has been described elsewhere.[19] Rates of biodegradation of the poly-alpha-hydroxyacids can be tailored by the polymer chemist so that the surgeon can vary the implant's application site. It may be possible to fabricate a rigid copolymer for internal bony fixation that will biodegrade

in consort with bony repair. It also may be possible to develop a spongy, semirubbery copolymer that will have application as a delivery source for bone induction agents that we feel have far more potential in the field of osseous repair and augmentation than do the PL's.

The positive bone healing response engendered by the copolymer-PL may be hypothesized to be a consequence of several factors. A unique chemical environment for calcium and phosphate precipitation, nucleation, and subsequent crystal growth is associated with PL's. Moreover, the PL has been described as being tantamount to a surrogate extracellular matrix vesicle, the structure whose limiting membrane is heavily endowed with a PL component.[13, 16, 17] The extremely important functions of matrix vesicles in the calcification phenomenon have been reviewed at length.[20-22]

Poly-alpha-hydroxy acids of the type described in this study are linear polyester macromolecules whose structure could serve as a matrix, trellis, or foundation upon which bony reparative elements may be consolidated.[17] The importance of collagen in bone formation has been attributed to its geometry and surface charge.[23, 24] There may be a parallel affect from the linearly arranged macromolecules comprising the copolymer of PLA:PGA.

Furthermore, as the copolymer degrades by nonspecific hydrolytic scission of its ester bonds, there is a shift in the local pH. Such an alteration could affect calcification inhibitors (i.e., proteoglycans, glycosaminoglycans) and could act in a beneficial manner to promote release from the host bone extracellular matrix of certain polypeptides, such as bone morphogenetic protein and human skeletal growth factor.[25]

We believe that the biodegradable polymers (of which PLA and PGA are two examples) offer a viable method for carriers of bone repair agents. Despite the fact that PL's are calcification inducers rather than ossification inducers, experimental defects did heal more rapidly when this agent was used in the polymer.[16, 17] However, the authors view the copolymer-PL combination as merely being of preliminary importance. The next phase of our maxillofacial bone repair alloplastic polymers will be studied in combination with ossification inducing agents which we believe will be of tremendous benefit to orthopedic and maxillofacial surgeons.

## CONCLUSION

Histomorphometric, clinical, and radiographic techniques were used to evaluate bony healing at discontinuity defects in dogs' mandibles that were treated with either a copolymer-PL or that were untreated. The results of these evaluations suggest that the copolymer-PL combination was useful in stimulating osseous repair. Moreover, when using a dog mandible as a model system for assessing efficacy of a bone repair material, it is prudent to extract the teeth in the planned ablation site and to allow for at least six weeks for mucosal healing before preparing the resections.

## MILITARY DISCLAIMER

"Commercial materials and equipment are identified in this report to specify the investigative procedure. Such identification does not imply recommendation or endorsement, or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the Army Medical Department."

ANIMAL STATEMENT

"In conducting research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources National Research Council."

ACKNOWLEDGMENTS

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## LEGENDS

### Figures

1a. Following resections in the control sides, rigid, internal 'ixation was used for treatment.

1b. The experimental sides were treated with the implant material (secured with wire) and internal rigid fixation.

2. Preparation of microscopic slide from implant/control-host site.

3a. A 22 mm X 10 mm rectangular secured over evaluation site to ensure for standardization.

3b. A typical histologic field used for measuring TV (woven bone in upper half of field and cortex in lower half).

4. Using a digitizer board, cursor, and microscope with drawing tube, traces of bony trabeculae were fed into a computer and algorithms for deriving three-dimensionality were used to determine trabecular bony volume (TV).

5a. Osseous regeneration within 4 week implant.

5b. No evidence of osseous repair within 4 week ablation site.

6a. Marked evidence of osseous bridging at 8 weeks.

6b. No indication that a bony bridge has formed at 8 weeks.

7a and 7b. Both implant and control sites show bony repair across the defect.

8a and 8b. Bony bridge apparent at lower border of mandible, whereas in the control defect no osseous repair can be visualized radiographically.

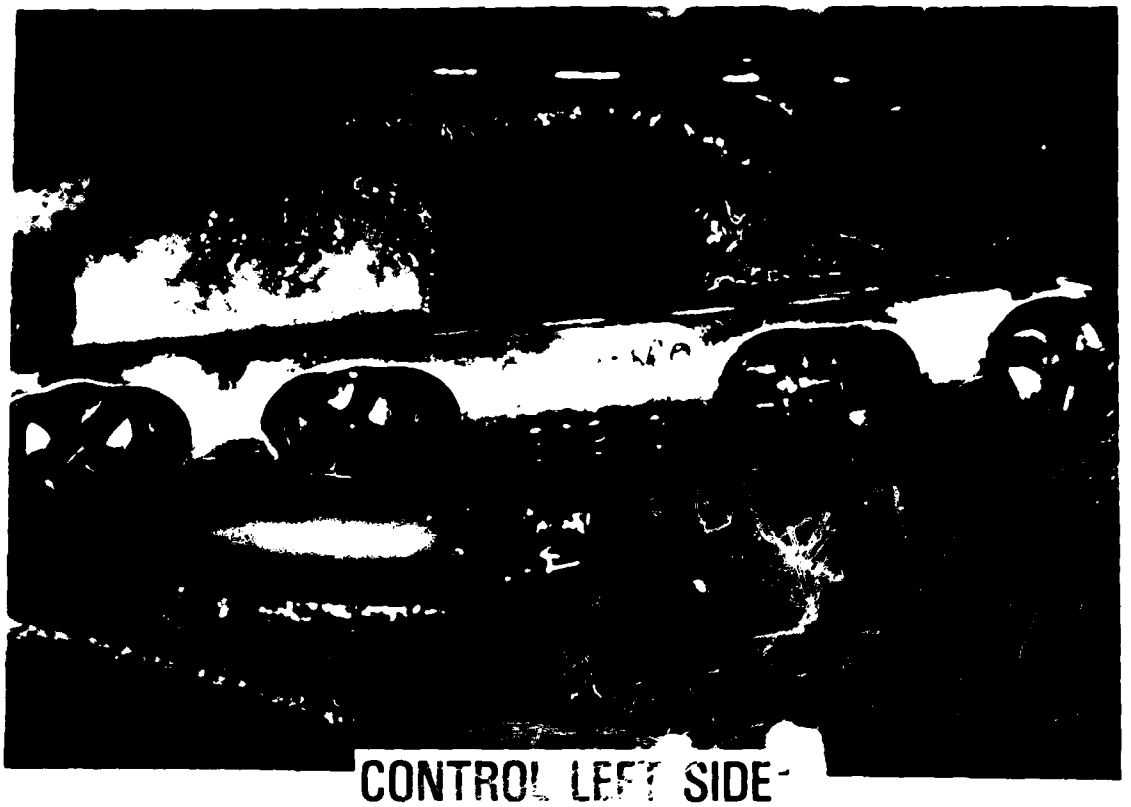
9a and 9b. Complete regeneration of the defect at the implant site by 40 weeks, while at the control site there is no evidence of bony repair.

10. Graph showing relationship of mean TV and standard error of the mean in implants and controls over the course of the experiment.

TABLE

Necropsy Schedule and Distributioin of Animals

| Temporal Groups (weeks) | 4 | 8 | 12 | 24 | 40 |
|---|---|---|---|---|---|
| Number of Animals | 5 | 5 | 5 | 5 | 5 |
| Extractions | 4 | 4 | 3 | 3 | 4 |
| No Extractions | 1 | 1 | 2 | 2 | 1 |



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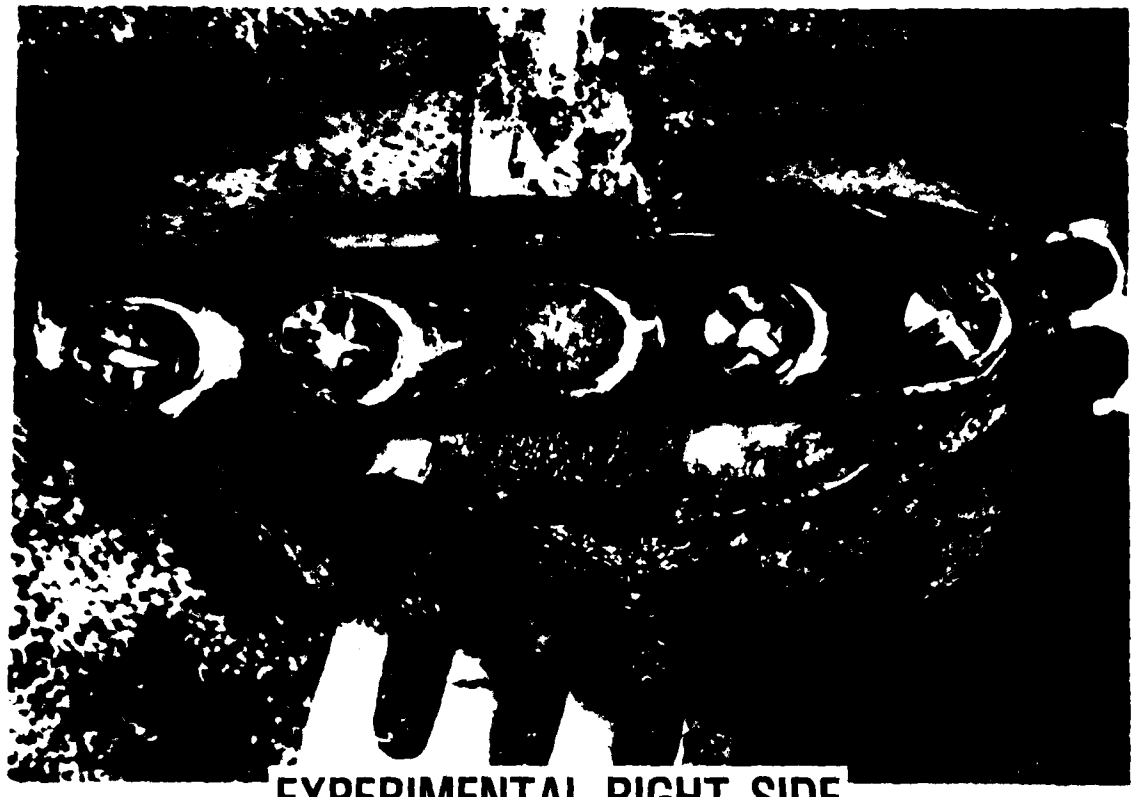
CONTROL LEFT SIDE



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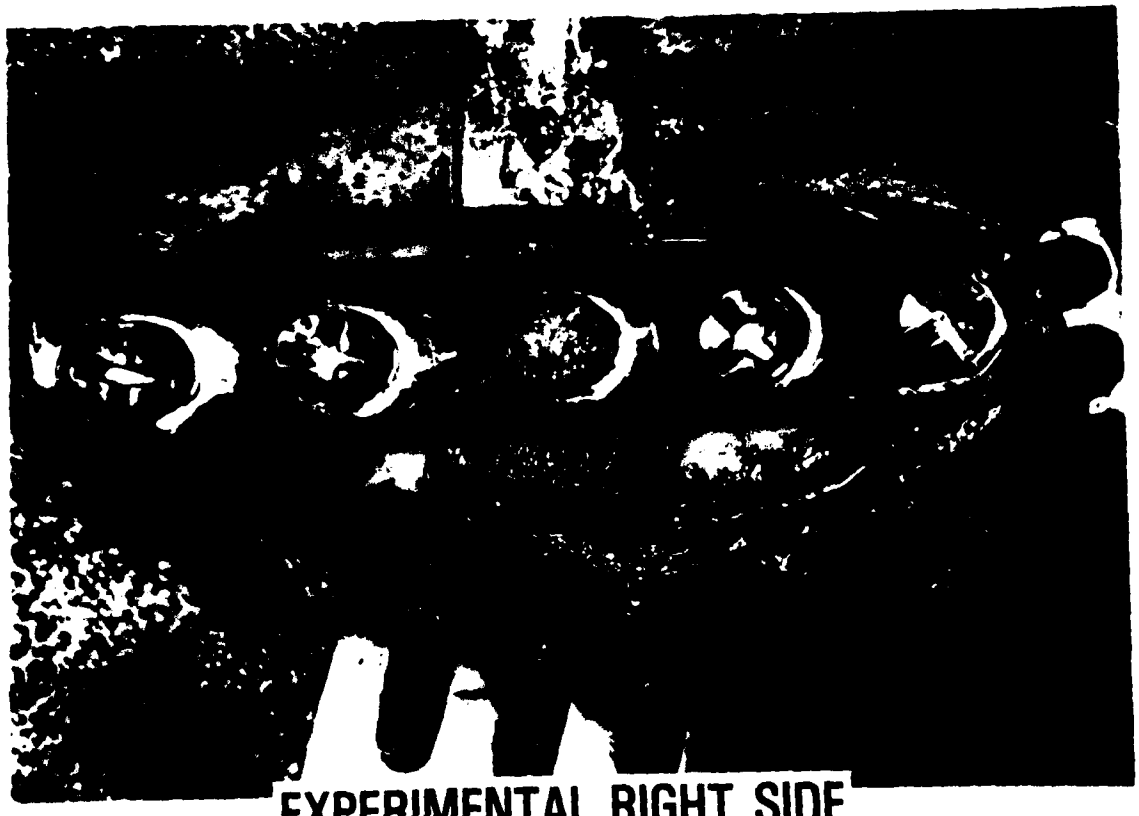

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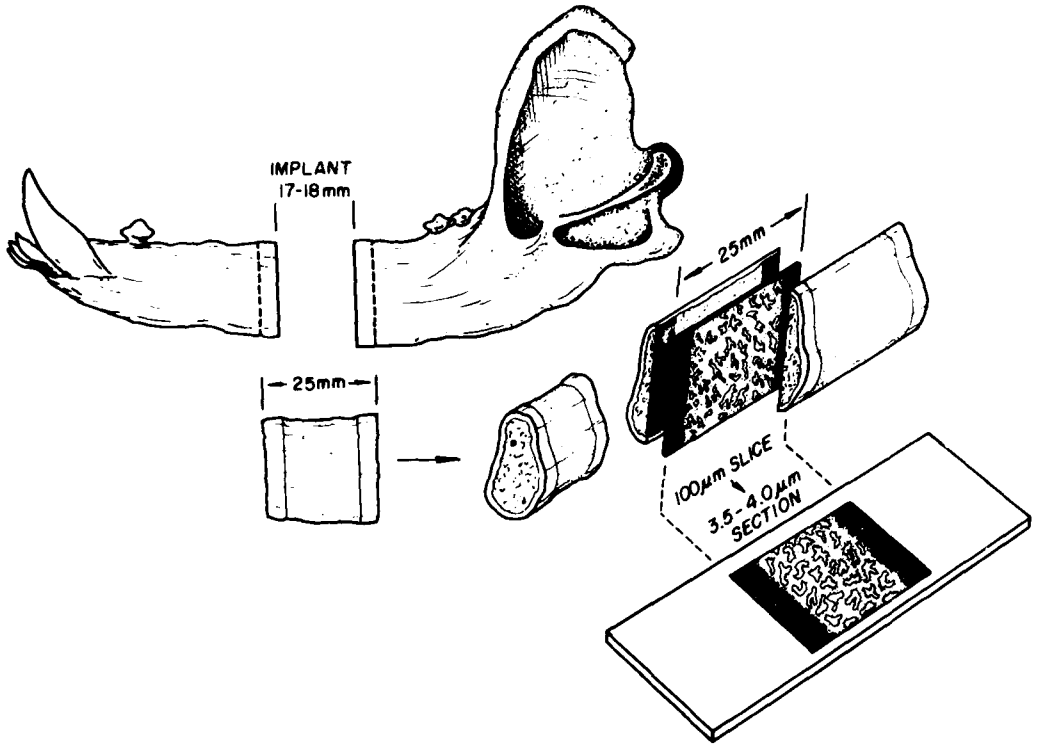
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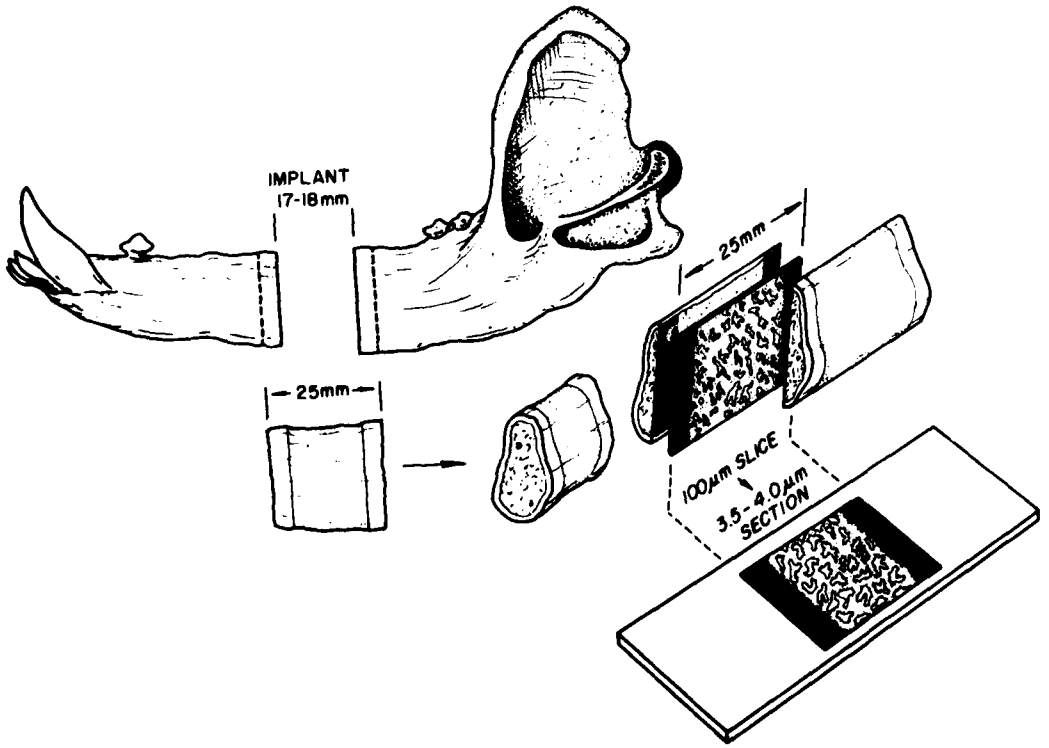
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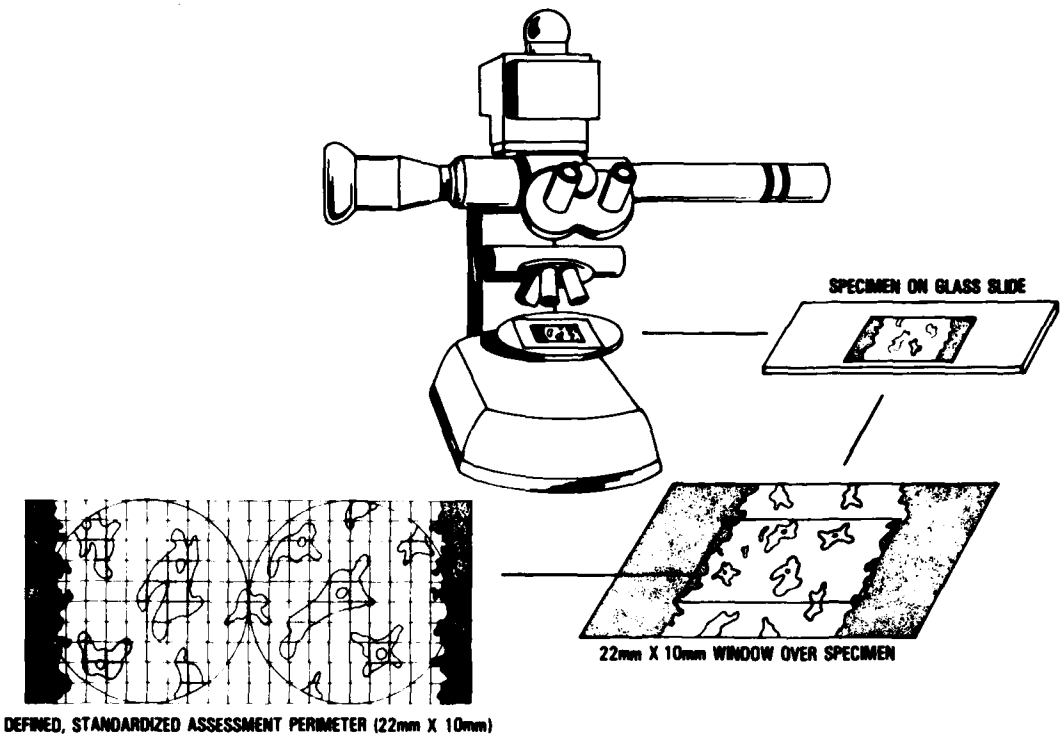


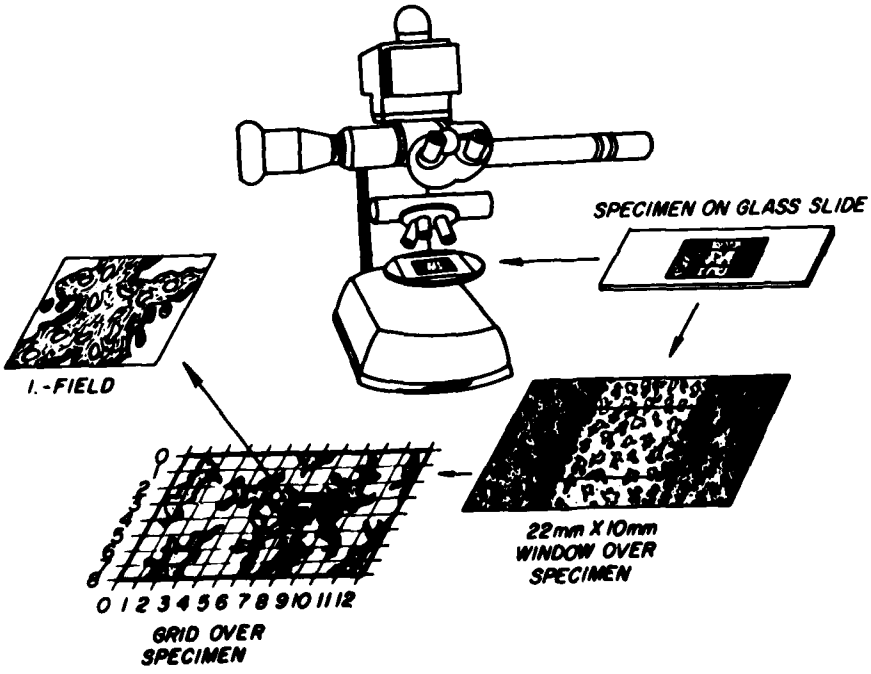


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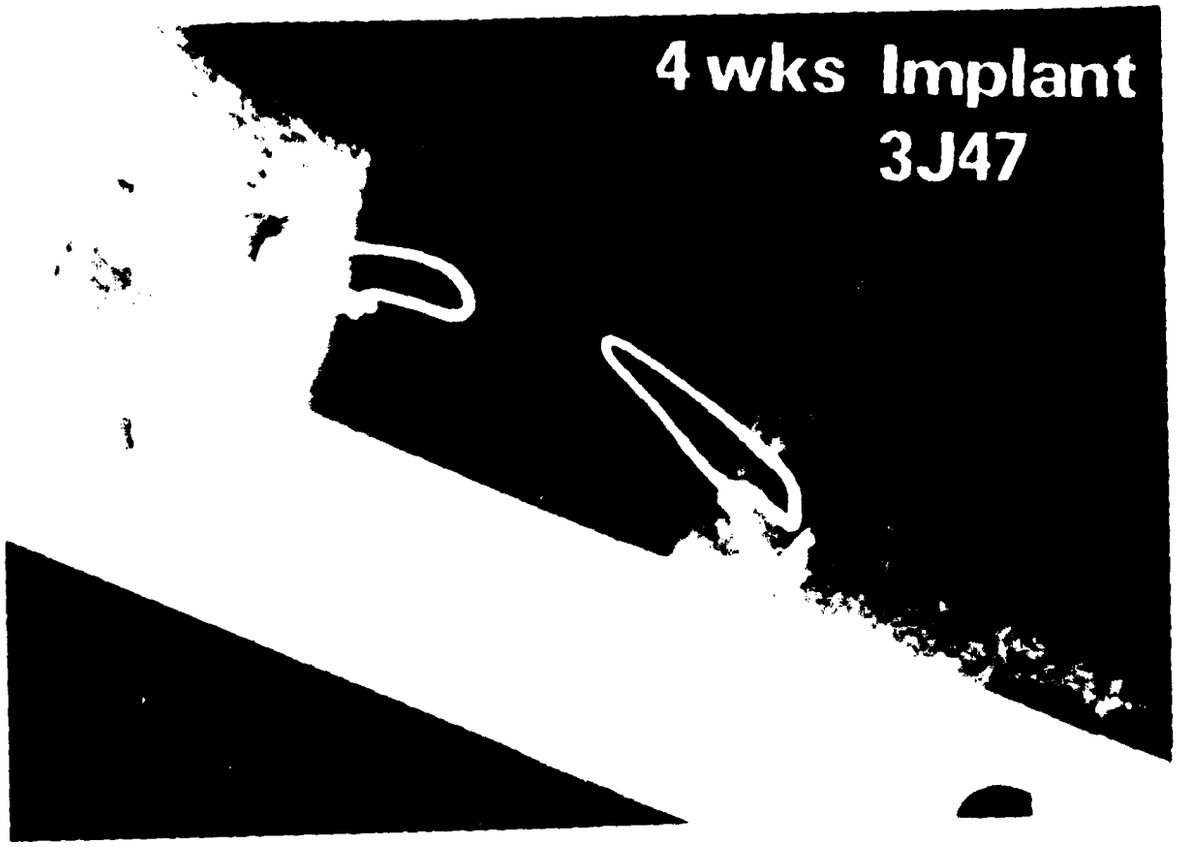


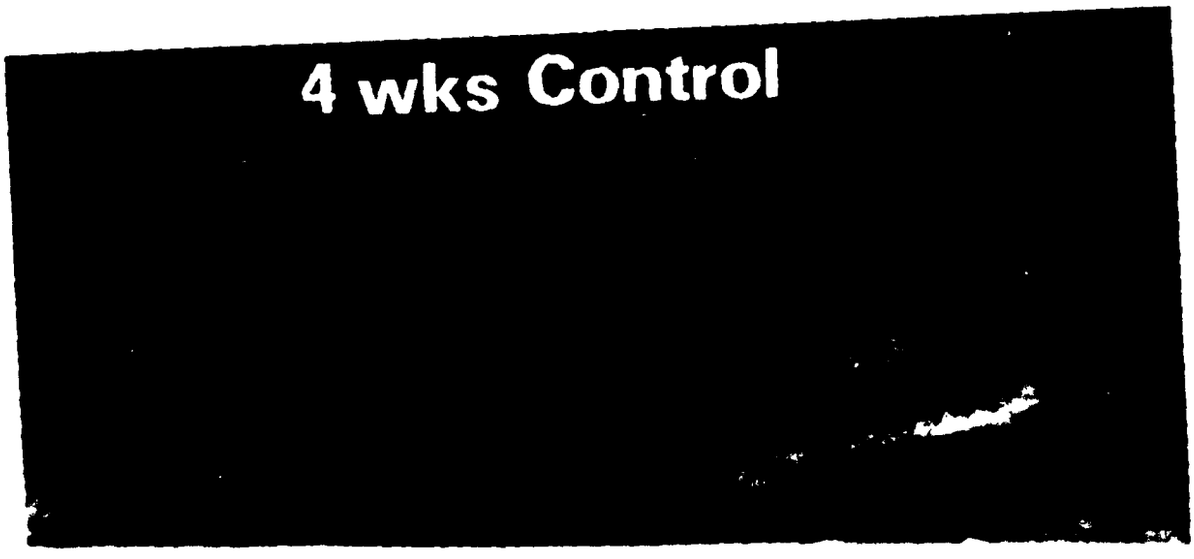


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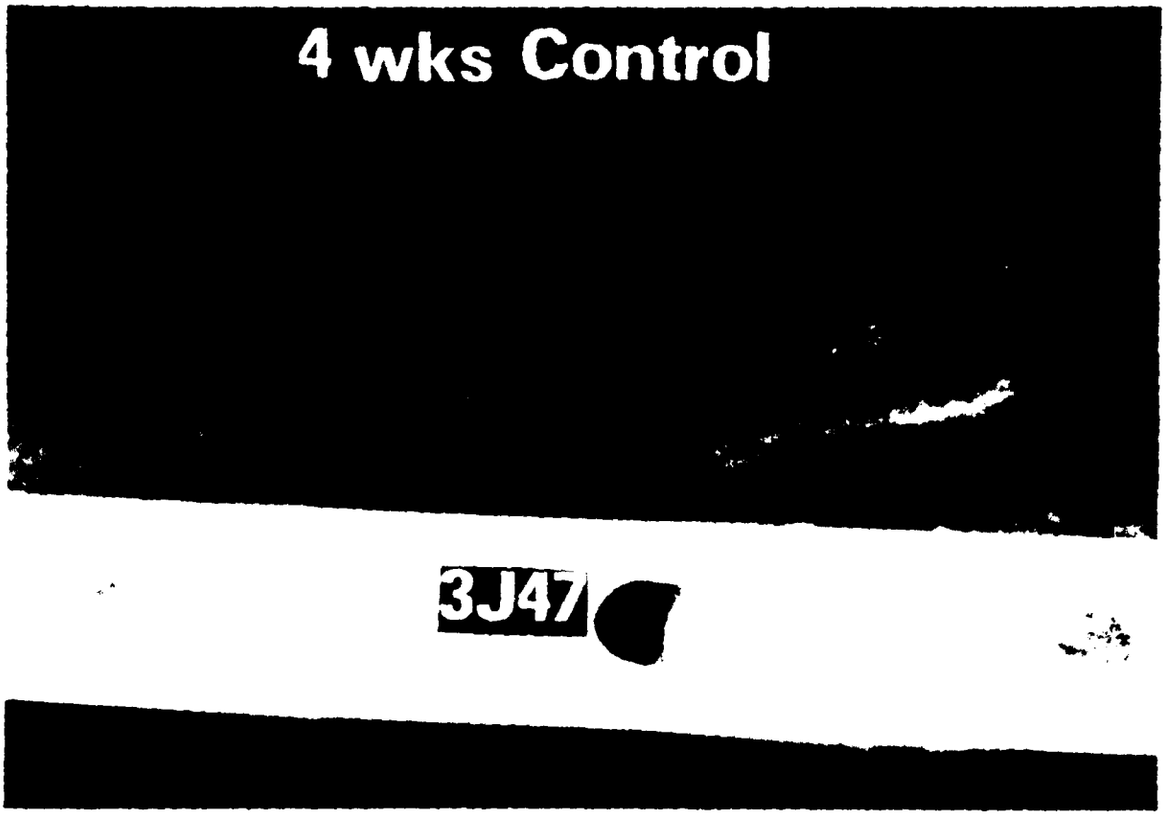
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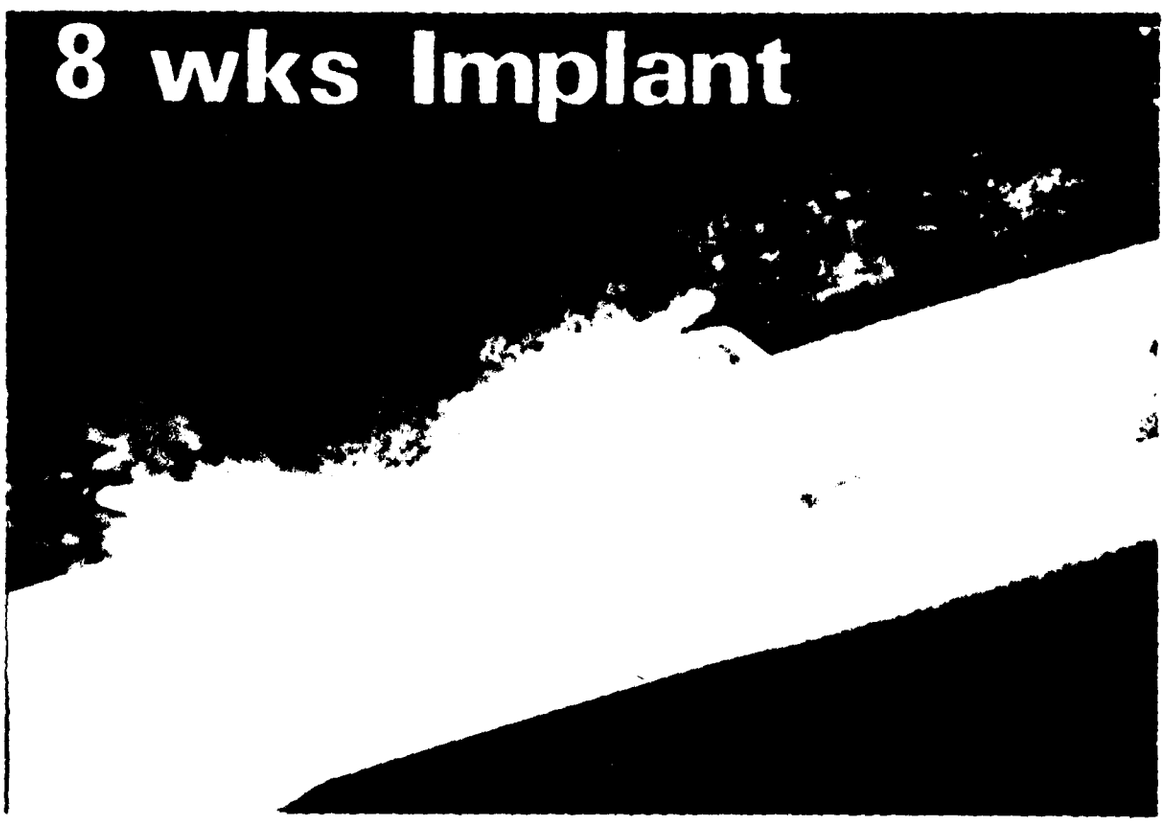
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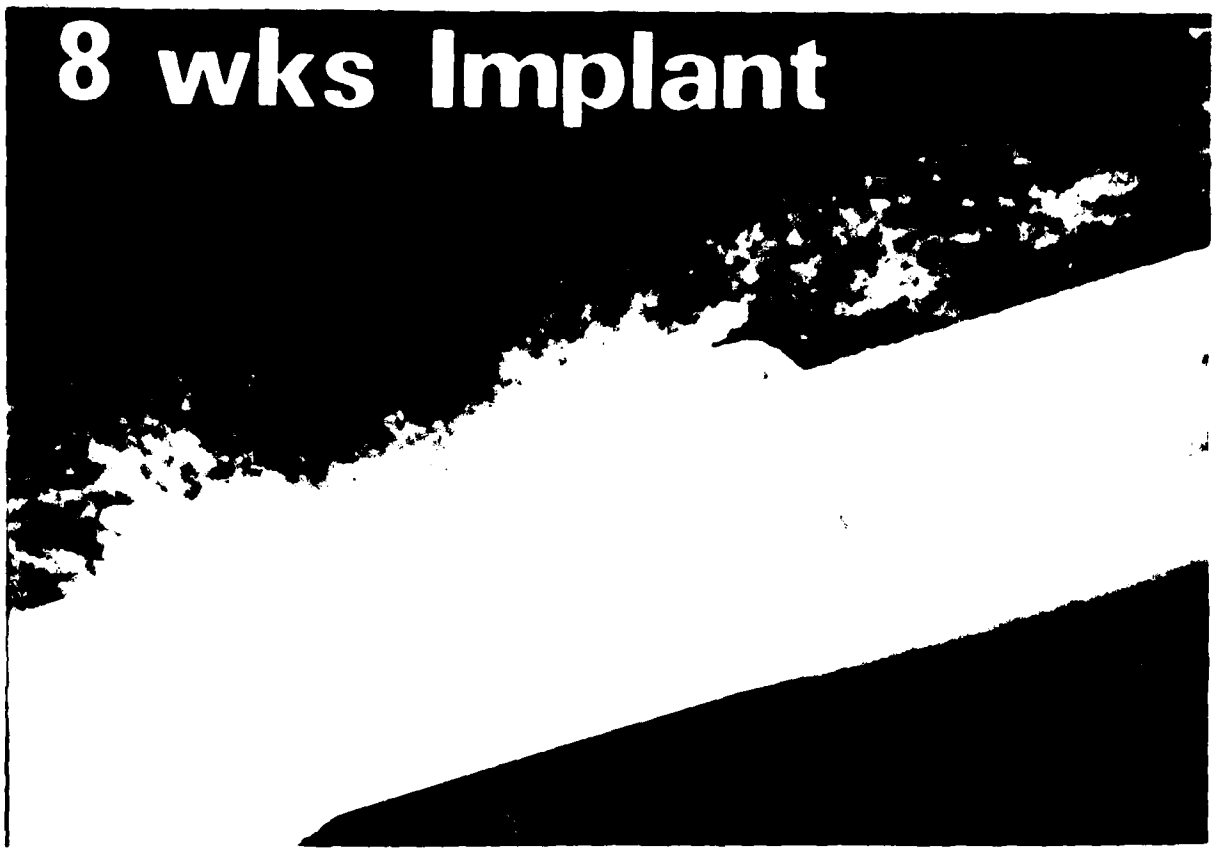
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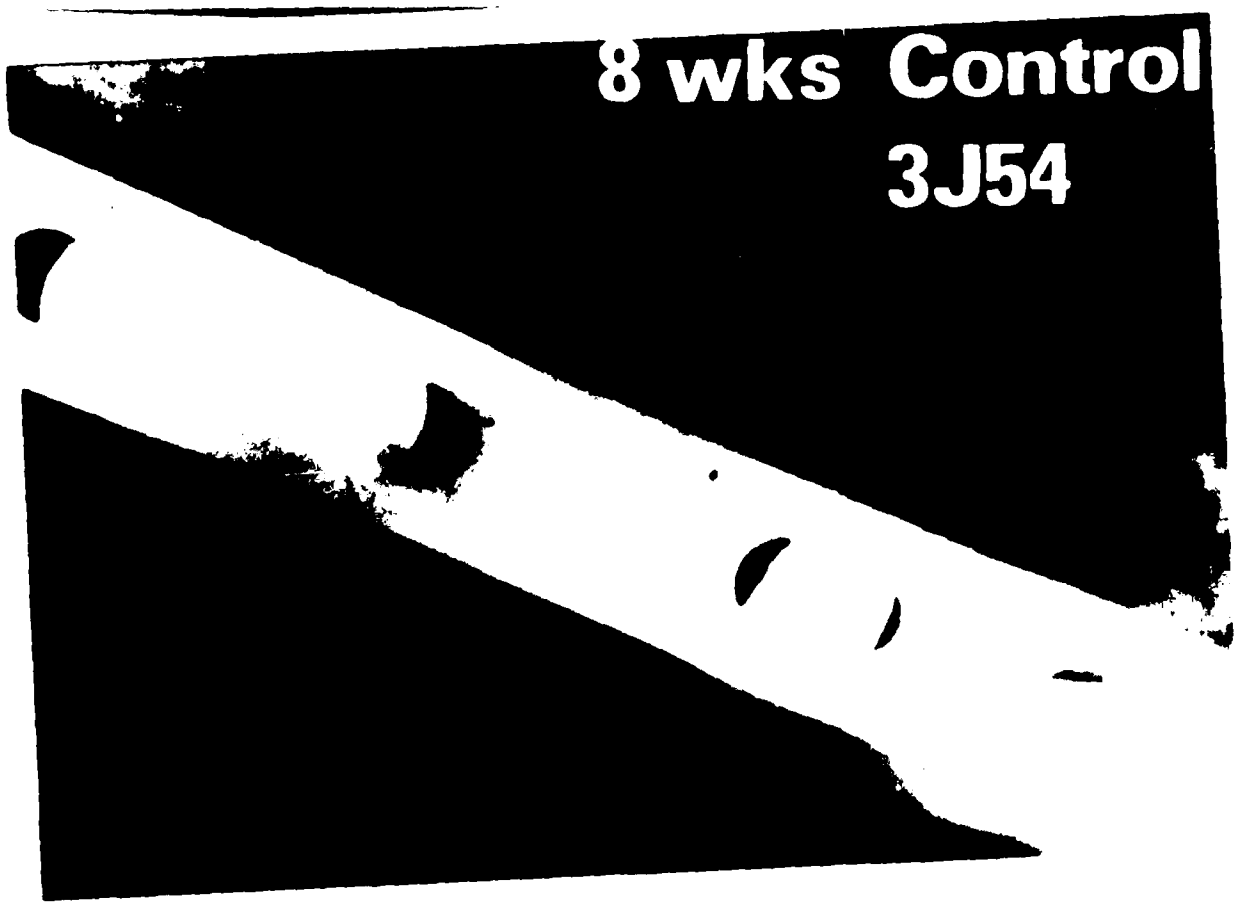
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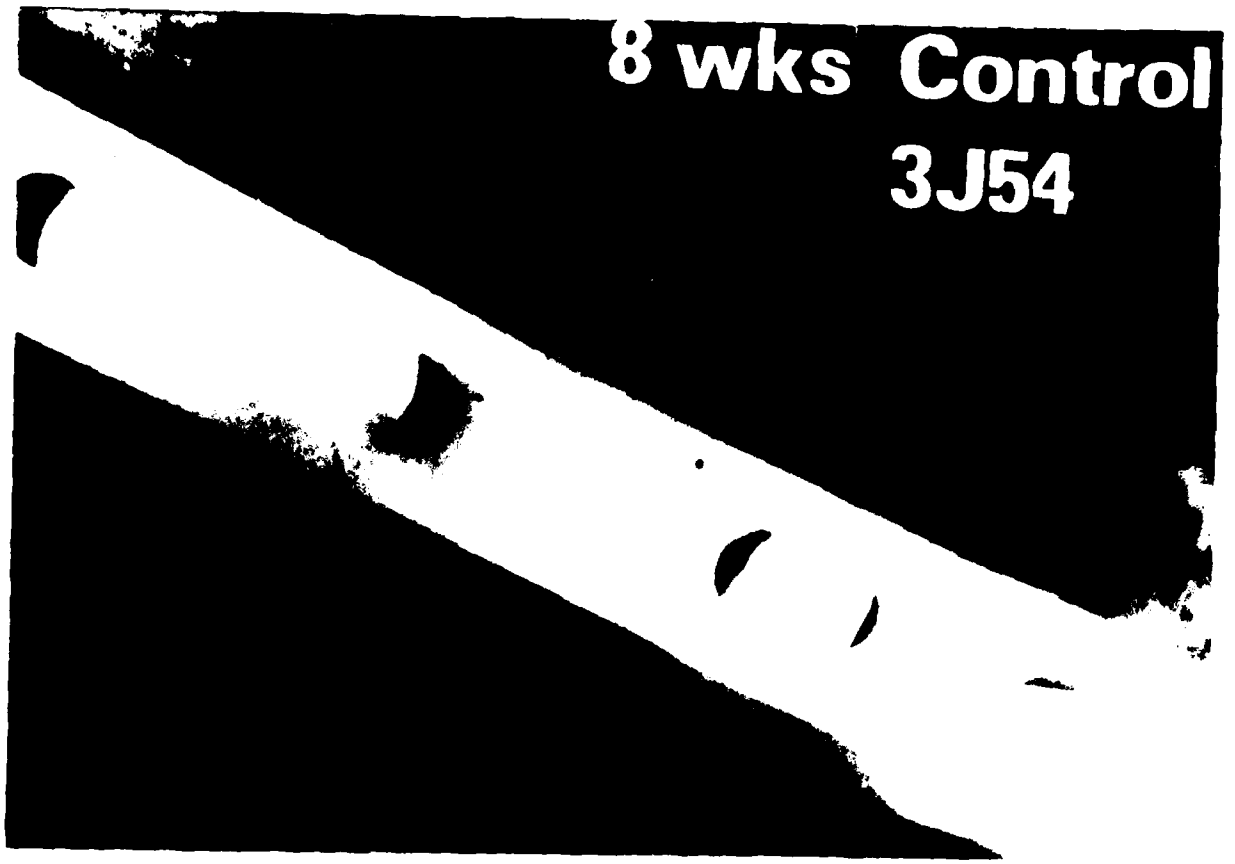
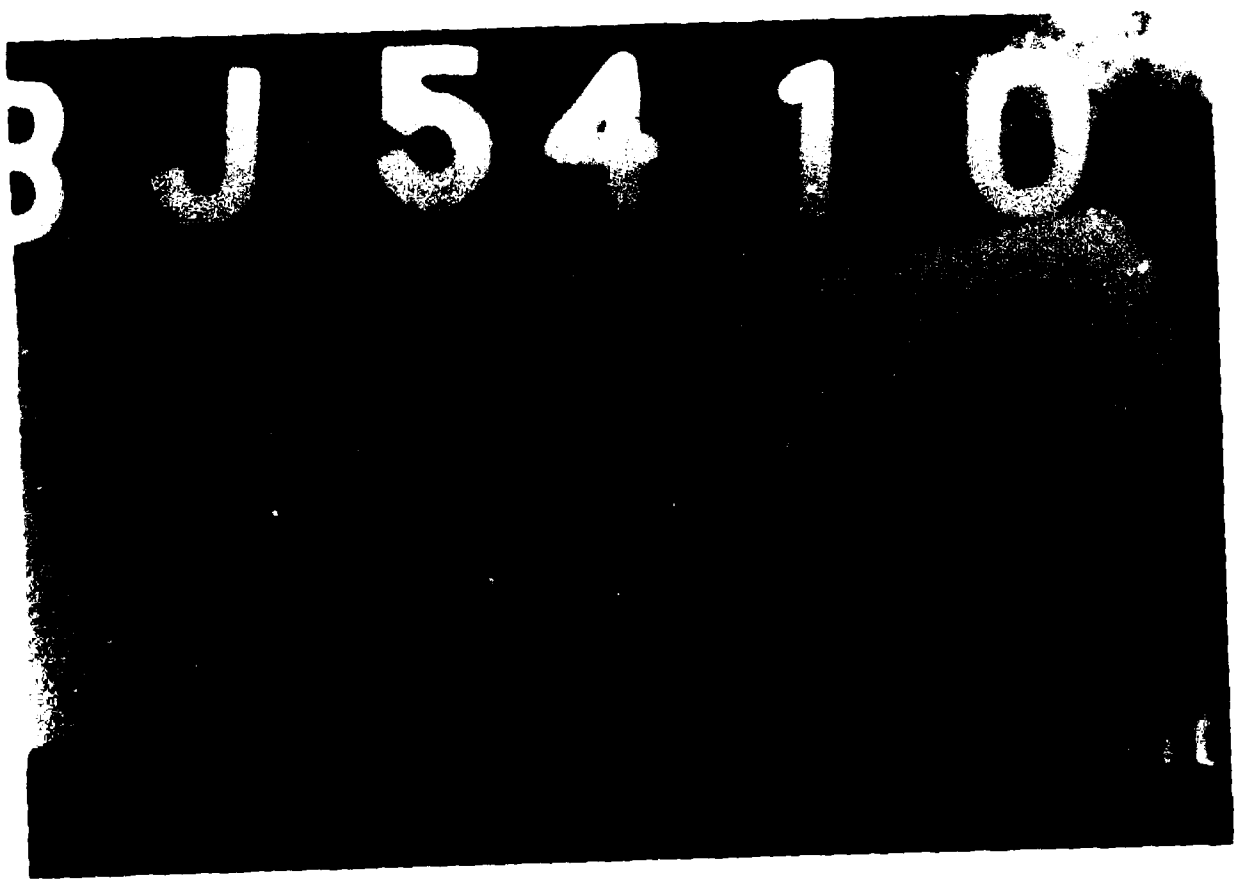
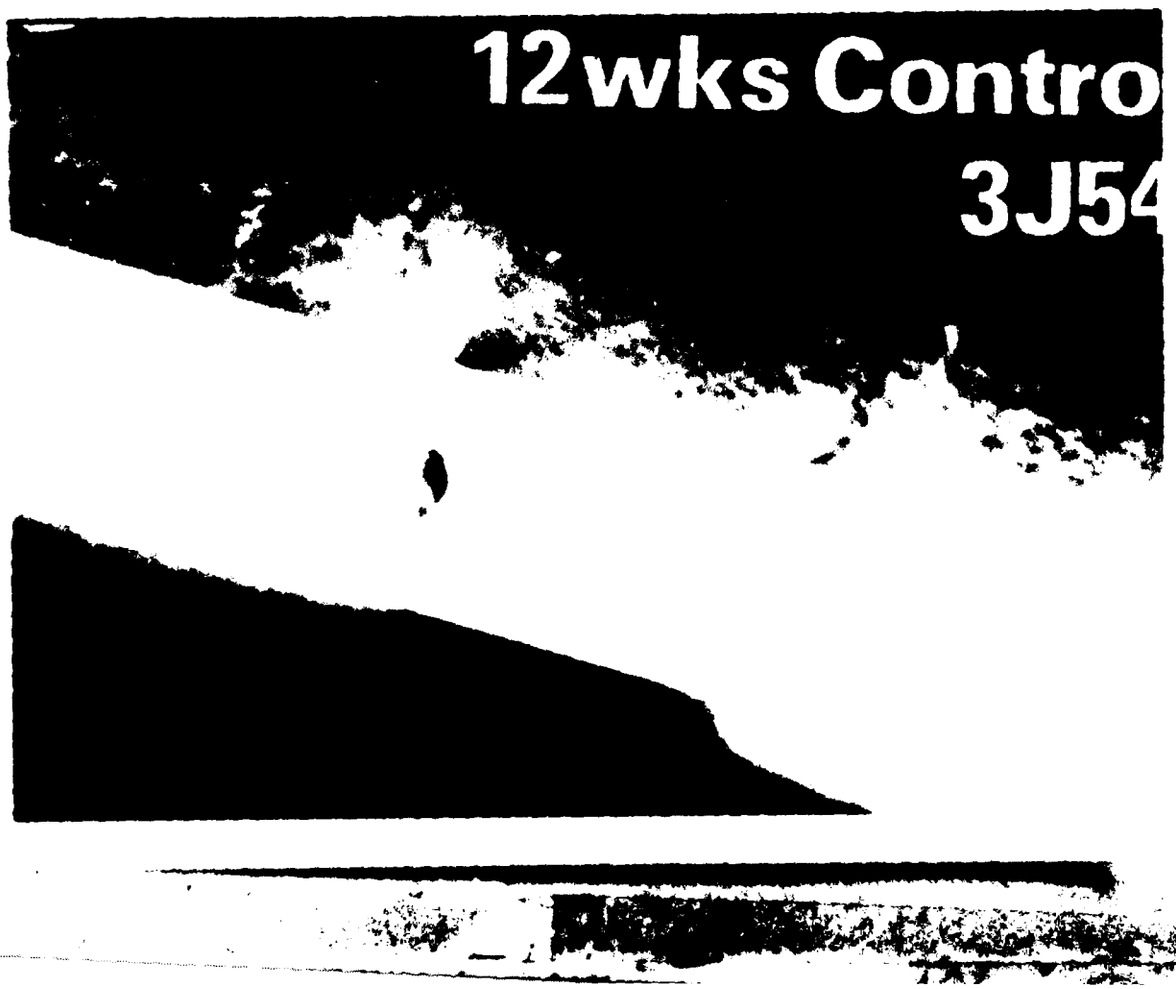
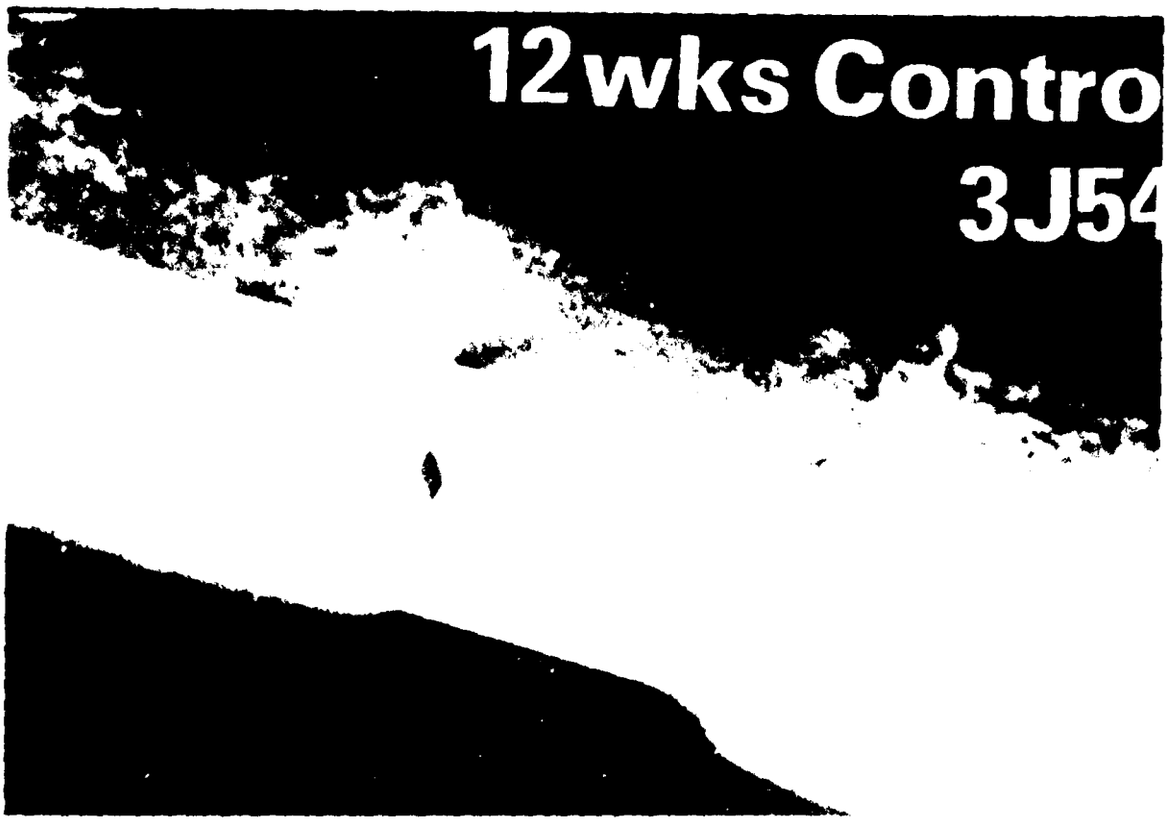

8 wks Control
3J54


3J5410


3J5410


12wks Contro
3J54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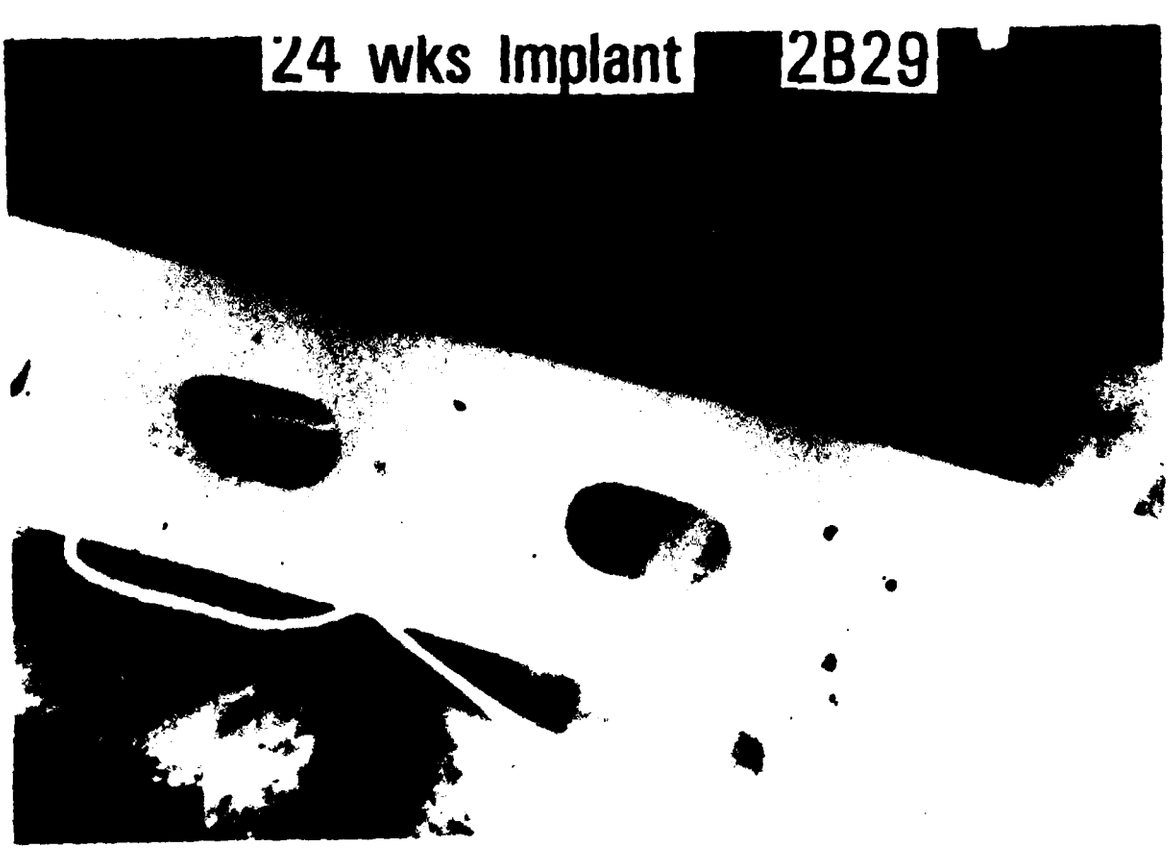
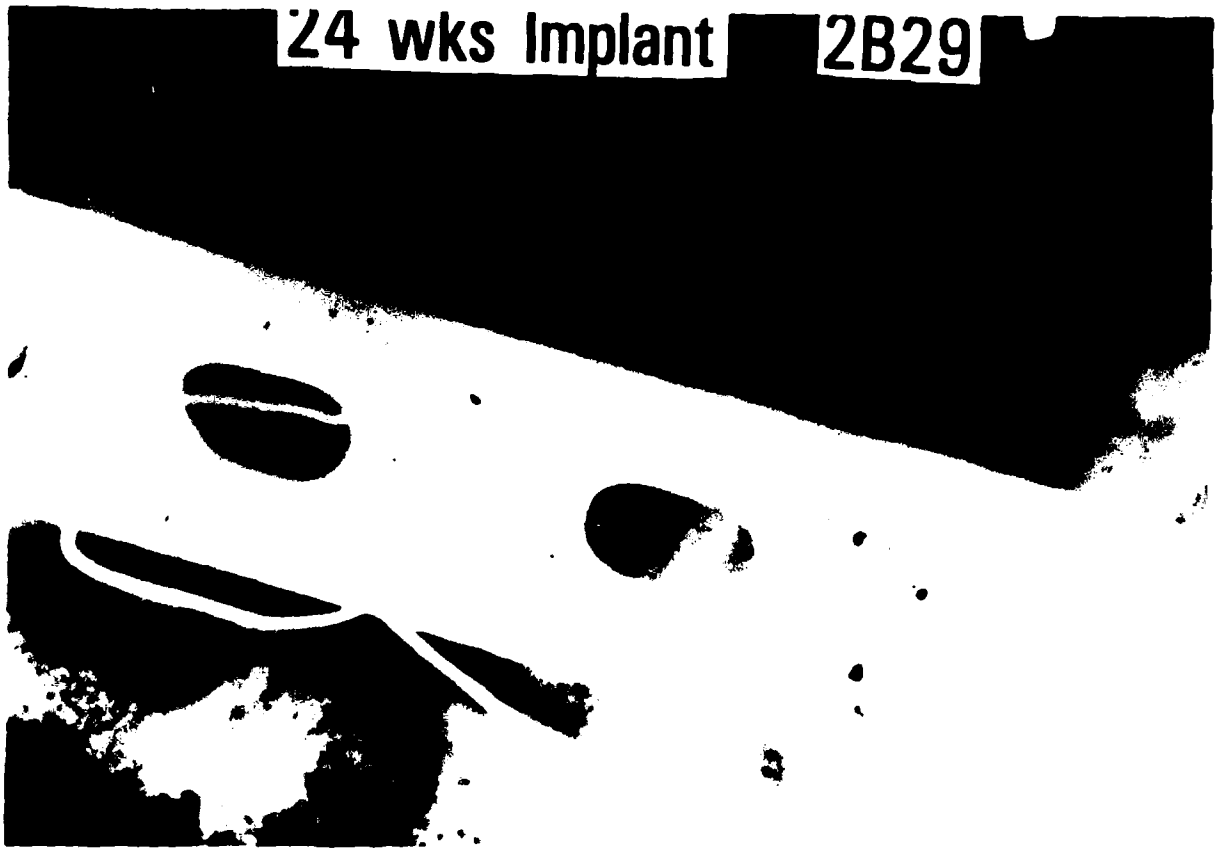

24 wks Implant
2B29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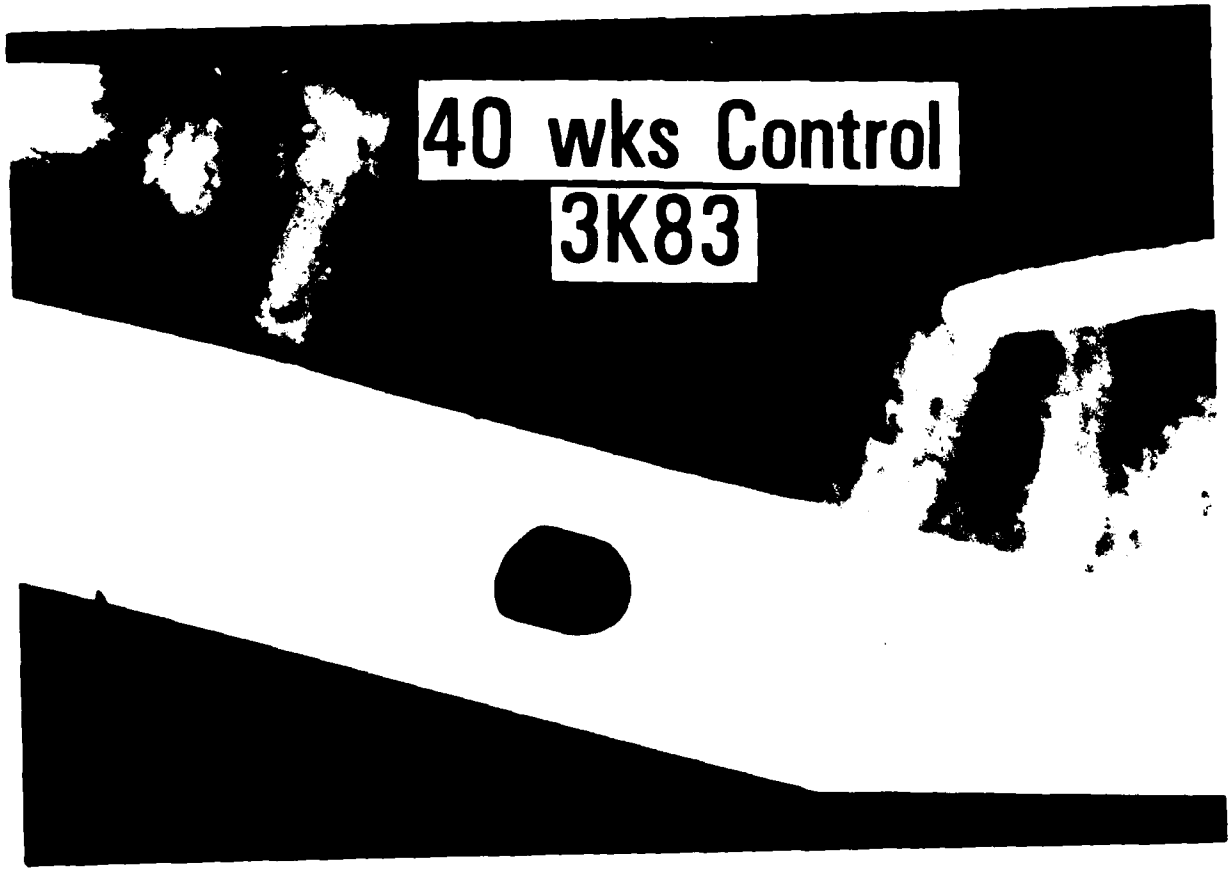
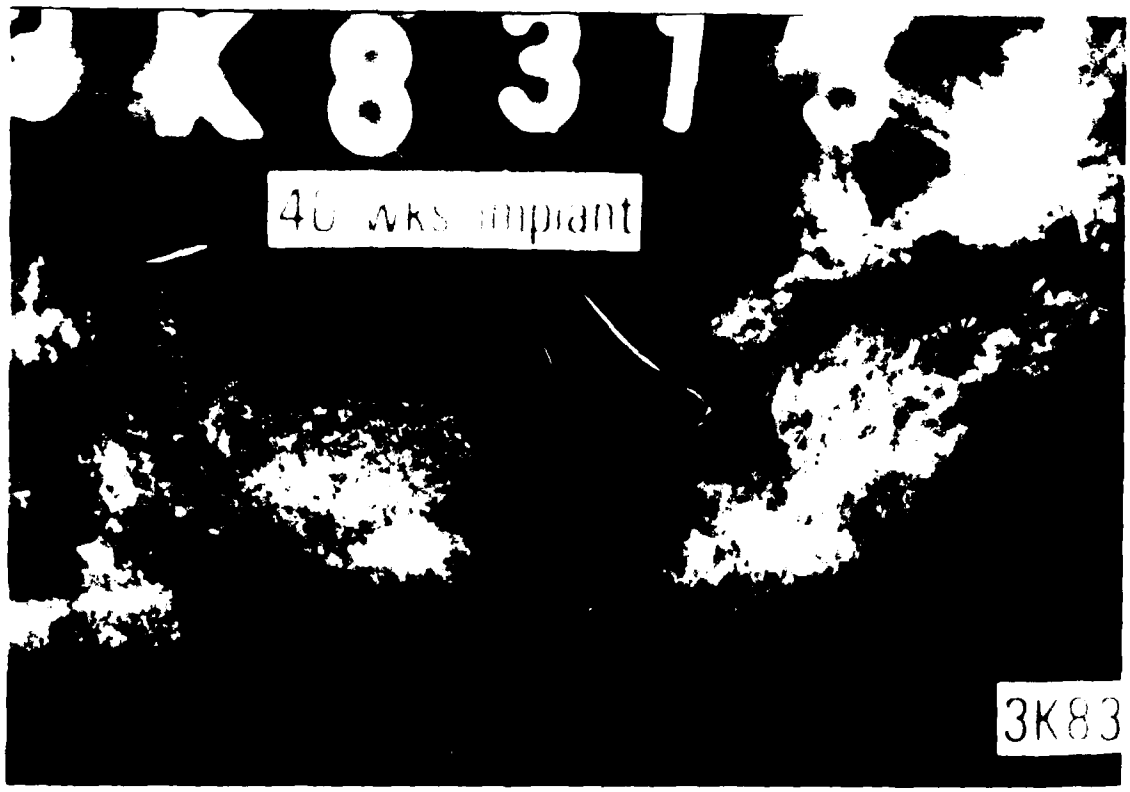
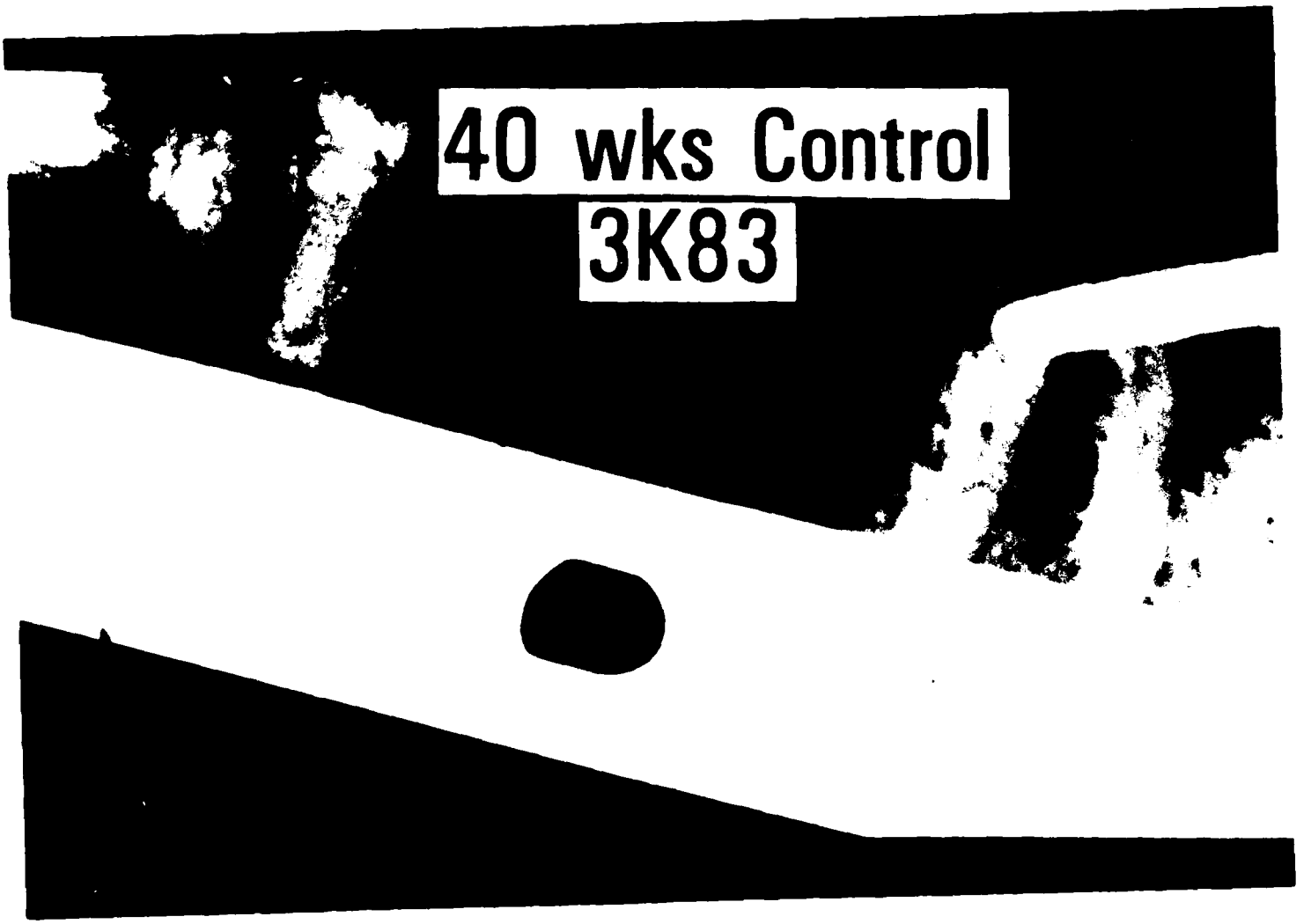

40 wks Control
3K83


3K831
40 wks implant
3K83


3K831
40 wks Implant
3K83


40 wks Control
3K83